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Rats infected with Streptococcus pneumoniae were used to study the effect of pneumococcal infection on the hepatic concentrations and the subcellular distribution of carnitine and coenzyme A (CoA). Compared to fasted control rats, fasted-infected rats have a decreased ketogenic capacity that is associated with an accumulation of total hepatic carnitine and a decrease in total hepatic coenzyme A. The concentration of competing substrates (e.g., lactate and pyruvate) and nucleotide ratios (e.g., [Acetyl-CoA]/[CoA], [ATP]/[ADP]·[HPO_4^{2-}], and [NAD^+]/[NADH]) are also variably affected by the infection. The

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concentrations of both lactate and pyruvate and the mitochondrial ratio of $[NAD^+]/[NADH]$ increased, while mitochondrial [acetyl-CoA]/[CoA] and the phosphorylation state or the $[ATP]/[ADP][HPO_4^{2-}]$ ratio decreased compared to fasted controls.

Since carnitine and CoA, the two key cofactors in fatty acid metabolism, are compartmentalized within the liver cell, the decreased ketone production observed during infection could be related to alterations in mitochondrial and cytosolic pools of these cofactors. Liver fractionation studies showed that in both fasted and fasted-infected rats, 6% of the total carnitine and 30% of the total CoA was in the mitochondria-rich fraction. During infection cytoplasmic acid-soluble carnitine and acetyl-CoA increased, while long-chain acylcarnitine and free CoA decreased compared to fasted controls. The mitochondrial pool size of carnitine was not affected by infection, but there was a 65% decrease in acetyl-CoA and a 50% decrease in free CoA. The carnitine acylation ratio reflected a decreased rate of fatty acid oxidation and the mitochondrial ratio of $[acetyl\text{-}CoA]^2$ to CoA suggested a decrease in precursor for ketone body synthesis during infection.

From these data and previous studies on esterification, it can be concluded that the infection-related decrease in ketone body production from long-chain fatty acid is due to an increased production of triglyceride from long-chain fatty acyl groups and an increased rate of shuttling of acetyl groups to carnitine and into the cytosol for the biosynthesis of new fatty acids and cholesterol.

Hepatic Concentration and Distribution of Coenzyme A and Carnitine during a *Streptococcus pneumoniae* infection in the Rat: Possible Implications on Fatty Acid Metabolism and Ketogenesis

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Preliminary reports of this study were presented at the Annual Meeting of the American Society of Biological Chemists in April 1979. Fed. Proc. 38:354, April 1979 and the American Federation of Clinical Research, in October 1978, Clin. Res. 26:627A. Excerpts have been taken from a dissertation submitted to the George Washington University Graduate School, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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ABSTRACT Rats infected with Streptococcus pneumoniae were used to study the effect of pneumococcal infection on the hepatic concentrations and the subcellular distribution of carnitine and coenzyme A (CoA). Compared to fasted control rats, fasted-infected rats have a decreased ketogenic capacity that is associated with an accumulation of total hepatic carnitine and a decrease in total hepatic coenzyme A. The concentration of competing substrates (e.g., lactate and pyruvate) and nucleotide ratios (e.g., [Acetyl-CoA]/[CoA], [ATP]/[ADP][HPO_4^{2-}], and [NAD^+]/[NADH]) are also variably affected by the infection. The concentrations of both lactate and pyruvate and the mitochondrial ratio of [NAD^+]/[NADH] increased, while mitochondrial [acetyl-CoA]/[CoA] and the phosphorylation state or the [ATP]/[ADP][HPO_4^{2-}] ratio decreased compared to fasted controls.

Since carnitine and CoA, the two key cofactors in fatty acid metabolism, are compartmentalized within the liver cell, the decreased ketone production observed during infection could be related to alterations in mitochondrial and cytosolic pools of these cofactors. Liver fractionation studies showed that in both fasted and fasted-infected rats, 6% of the total carnitine and 30% of the total CoA was in the mitochondria-rich fraction. During infection cytoplasmic acid-soluble carnitine and acetyl-CoA increased, while long-chain acylcarnitine and free CoA decreased compared to fasted controls. The mitochondrial pool size of carnitine was not affected by infection, but there was a 65% decrease in acetyl-CoA and a 50% decrease in free CoA. The carnitine acylation ratio reflected a decreased rate of fatty acid oxidation and the mitochondrial ratio of $[\text{acetyl-CoA}]^2$ to CoA suggested a decrease in precursor for ketone body synthesis during infection.

From these data and previous studies on esterification, it can be concluded that the infection-related decrease in ketone body production from long-chain fatty acid is due to an increased production of triglyceride from long-chain fatty acyl groups and an increased rate of shuttling of acetyl groups to carnitine and into the cytosol for the biosynthesis of new fatty acids and cholesterol.

INTRODUCTION

Rats infected with Streptococcus pneumoniae have a decreased hepatic ketogenic capacity which is associated with an accumulation of carnitine (1) and a decrease in CoA[1] fatty acyl esters (2). The production of ketone bodies from lipid stores is thought to be an important metabolic response to fasting. This reduced ketogenic ability of the fasted-infected host could contribute to the marked catabolic response of the host by promoting the continued use of amino acids for gluconeogenesis.

Previous studies also suggested that during infection there is a redistribution of fatty acid between the pathways of esterification and oxidation (1, 2). Activation of long-chain fatty acids to acyl-CoA esters occurs primarily in the cytoplasm (3), as does the biosynthesis of complex lipids (4). However, the enzymes which catalyze beta-oxidation of long-chain fatty acids are localized within the mitochondrial matrix and peroxisomal spaces (5), while ketone synthesis occurs only in the matrix. Therefore, since triglyceride synthesis, oxidation and ketogenesis are compartmentalized processes, control is dependent on localized changes in the concentration of intermediates within each compartment.

Infection-related alterations in the subcellular concentrations of hepatic carnitine and coenzyme A, may be indicative of changes that could affect the rate of flux through these compartmentalized processes. Carnitine is located primarily in the cytosol but is exchanged freely with mitochondrial carnitine (6). A greater percentage of the total hepatic CoA content is mitochondrial (7), but the inner membrane of the mitochondria forms a permeability barrier to CoA and CoA esters, thereby creating two separate nonexchangeable CoA pools (7). The purpose of the present study was to determine the intracellular distribution of CoA, carnitine and their derivatives in livers from fed, fasted and fasted-

infected rats. Quantitating the changes occurring in metabolite concentrations within the various cellular compartments could lead to a better understanding of the mechanisms that regulate hepatic rates of fatty acid oxidation and ketone production.

METHODS

Animals. Male, Fisher-Dunning rats, F-344/Mai J (Microbiological Associates, Walkersville, Md.), weighing 175-250 g, were used in all experiments. Rats were adapted over a 2-week period to an 0800-1000 hours meal-feeding of a standard diet (Teklad Test Diets, Division of ARS/Sprague-Dawley, Madison, Wis.) which consisted of 10% fat, 26% protein, and 60% carbohydrate. Rats were maintained in a light- and temperature-controlled room [12 h light (1000-2200 hours) and 12 h dark, 23 $\pm$ 1°C] during the experiment.

After 2 weeks on the above feeding schedule 10 rats were killed at 1000 hours and the remainder were inoculated subcutaneously with 0.1 ml of a saline-diluted culture containing 1-3 x 10^4 live or heat-killed (56°C, 20 min) S. pneumoniae. Rats receiving the heat-killed organisms served as controls. All rats were then fasted and kept in wire-bottom cages and allowed water ad libitum. Subsequent body temperature was measured by rectal probe (Yellow Springs telethermometer, Yellow Springs, Ohio). Details concerning preparation of inocula and the clinical manifestations of the infection have been published elsewhere (8). At 48 h postinoculation, 10 rats from each group were killed by cervical dislocation. Within 10 sec after killing, the livers were removed and crushed between liquid nitrogen cooled aluminum blocks. Blood which had accumulated in the chest cavity after severing the vena cava was collected in heparinized tubes.

In other experiments livers from fed, fasted and fasted-infected rats were perfused by a modification of the technique of Miller (9) as previously described (10). The perfusion medium consisted of 70 ml Krebs-Ringer bicarbonate buffer (pH 7.2), 30 ml washed sheep erythrocytes, 3 g fatty acid-poor bovine serum albumin (Calbiochem) and 500 U of

heparin. The rate of perfusion was maintained at 1 ml/min g^{-1} and the perfusate was recirculated. At the end of a 60-min equlibration period, 2.5 ml of a 20 mM solution of either oleic acid- or octanoic acid-albumin complex (1) was added to the perfusate and the same fatty acid complex was infused thereafter at the rate of 6 ml/h. At the end of the 60-min infusion of labeled fatty acid, the livers were crushed between liquid nitrogen-cooled aluminum blocks.

Free carnitine, short-chain acylcarnitines (11), CoASH and acetyl-CoA (12) were measured in a neutralized extract of liver. To approximately 1.5 g of powdered tissue was added an equal volume of 3% $HClO_4$. The tissue was homogenized and centrifuged for 10 min at 3,000 x g at 4°C. The pellet was washed twice with 1.5% $HClO_4$ and centrifuged after each washing. The last centrifugation was performed at 8,700 x g for 10 min. The supernatant and the washes were neutralized to pH 6-7 with 20% KOH.

Approximately 0.5 g of the pellet was hydrolyzed with 1.0 ml of 20% KOH at 50°C for 2 h. This hydrolysate was centrifuged, neutralized and assayed for long-chain acylcarnitines (11). Acyl-CoA was hydrolyzed enzymatically (13) from approximately 0.2 g of the acid extract pellet by first homogenizing in 4 ml of a mixture containing 10 ml potassium phosphate buffer (pH 6.8), 1 ml EDTA (0.05 M), 1 ml dithiothreitol (0.1 M), and 38 ml deionized water. This homogenate was neutralized with approximately 0.08 ml of 2 N KOH. One milliliter of the neutralized homogenate was incubated with 10 µl of activated fatty acid synthetase (14) for 40 min at 30°C. A 1-ml blank was treated with 0.1 ml 2 N HCl and kept on ice. The reaction was terminated by the addition of 0.1 ml HCl to the sample at 30°C. This sample was immediately placed on ice. Both sample and sample blank were centrifuged at 8,700 x g for 10 min.

The supernatant was neutralized with 0.12 ml of 1 N KOH and assayed for long-chain acyl CoA (12).

Malonyl-CoA was determined according to McGarry et al. (15). Hepatic 3-hydroxybutyrate and acetoacetate were measured as described by Neufeld et al. (16). Cytoplasmic and mitochondrial $[NAD^+]/[NADH]$ ratios were calculated as described by Williamson et al. (17). Malate and α-glycerophosphate were assayed by the method of Michal and Lang (18). Plasma was analyzed for free fatty acids, ketone bodies and insulin as previously described (1). Oxaloacetate, citrate, dihydroxyacetone phosphate, and the $[NADP^+]/[NADPH]$ ratio were calculated as described by Gumaa et al. (19). Adenine nucleotides were measured according to the method of Neufeld et al. (20).

Fractionation studies. Fed, fasted-control and fasted-infected rats were stunned by a blow to the head and decapitated. Blood was collected in chilled heparinized test tubes. The liver was removed, perfused with cold (4°C) 0.25 M sucrose until clear of visible blood, blotted and weighed. All subsequent fractionation procedures were carried out at 0-4°C.

A 10% (w/v) tissue homogenate was prepared with a Potter-Elvehjem homogenizer in a medium containing 220 mM mannitol, 70 mM sucrose, 5 mM morpholinopropane sulfonic acid (MOPS), and 2 mM EDTA (pH 7.4). Homogenates were centrifugated at 750 x g for 10 min resulting in a nuclear pellet and a cytoplasmic extract. The supernatant from the low-speed centrifugation was recentrifuged at 8,000 x g for 10 min and the pellet washed twice. The resulting pellet represents the large granule or mitochondria-rich fraction, while the supernatant and washes constitute the post-mitochondrial supernatant. The final mitochondria-rich pellet

was resuspended to a protein concentration of 40 mg/ml in the homogenizing medium without EDTA. Aliquots of each fraction were either assayed for carnitine, CoA, their derivatives and marker enzymes or were frozen (-70°C) for future analysis.

To test the integrity of the mitochondria in the large granule fraction, oxygen consumption experiments were performed using a Clark-type oxygen electrode in a 3-ml water-jacketed chamber at 30°C. The chamber contained 80 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM inorganic phosphate at pH 7.0 and 2-5 mg of mitochondrial protein. State 3 and State 4 (succinate) respiration (45 and 9 nmol O_2/min mg^{-1}, respectively, for the fasted preparation), the respiratory control ratio and the ADP/O ratio (7 and 1.9, respectively) were calculated as previously described (21). These results indicate that mitochondria were functional and in the coupled state.

Livers from another group of rats were further fractionated by differential centrifugation and isopycnic sucrose density centrifugation to separate peroxisomes and mitochondria. The methods were based on the procedures of Leighton (22) and Beaufay (23). Marker enzymes were measured as described previously (24-27). In these preparations the large granule fraction typically contained 48% of the total catalase (peroxisomal marker), 48% of the glutamate dehydrogenase (mitochondrial marker), 54% of the N-acetyl-β-glucosaminidase (lysosomal marker), 27% of the original protein, 11% of the 5'-nucleotidase (plasma membrane marker) and 13% of the NADPH cytochrome c reductase (endoplasmic reticulum marker) activities (28). Most of the glutamate dehydrogenase was found in the density region, 1.18-1.20, while peroxisomes equilibrated in the denser fractions of the gradient, 1.23-1.25. Aliquots of each fraction were used for the determination of CoA and carnitine.

The cellular content of mitochondrial protein in both studies was estimated by measuring the activity of glutamate dehydrogenase, a mitochondrial marker enzyme, in tissue homogenate, mitochondrial, nuclear and post-mitochondrial supernatant fractions. The mitochondrial content was calculated as described by Idell-Wenger et al. (29).

Statistical analysis. All data were analyzed by one-way analysis of variance. A probability value less than 0.05 was considered significant under the null hypothesis.

RESULTS

Rats that received 1-3 x 10^4 CFU of live S. pneumoniae had elevated rectal temperatures by 24 h postinoculation. By 48 h the body temperatures of some rats began to decline and 20% of the rats died between 46 and 52 h. Rats chosen for the present studies had body temperatures that remained elevated at 48 h. All infected rats not chosen for this biochemical study died by 72 h. All fasted-infected rats studied had elevated plasma insulin and decreased concentrations of plasma free fatty acids, ketone bodies and zinc. These parameters plus other clinical manifestations are consistent with published data concerning this infectious model (8). Fasted rats given the heat-killed organisms neither developed fever nor showed changes in plasma constituents; they served as additional fasted controls for each infected group.

In vivo and in vitro effects of infection on hepatic carnitine and CoA concentrations and subcellular distribution. Liver weight is known to decrease during starvation and increase during the acute stages of pneumococcal infection, while the DNA content remains constant (30). Therefore, where appropriate, results are also expressed on a total liver basis to correct for these weight changes.

Both acid-soluble and insoluble carnitines increased in livers from rats infected with S. pneumoniae compared to fasted control livers (Table I). Total carnitine (acid-soluble + insoluble) increased in liver from 48-h, fasted-infected rats whether expressed on a per gram of liver or total liver basis (fed, 1736 $\pm$ 138; fasted, 1552 $\pm$ 57; fasted-infected, 3747 $\pm$ 172 nmol/liver). When perfused with an oleic acid substrate, livers from fasted rats produced ketones at a faster rate than those from fed or fasted-infected rats (2.18 $\pm$ 0.19 vs. 0.64 $\pm$ 0.10 vs. 1.10 $\pm$ 0.10 µmol/min per 100 g BW, respectively). No differences were

observed in the rates of ketogenesis from octanoic acid during infection. In neither group was there a difference in the amount of fatty acid taken up by the livers (1, 2). The data in Table I indicate that during infusion with oleic acid the concentration of free carnitine was slightly elevated by the infection. However, the concentration of short-chain acylcarnitines, mainly acetylcarnitine, and long-chain acylcarnitines, increased in perfused livers from infected rats compared to fasted control rats. No change occurred in the carnitine content per liver during the 48-h fast (fed, 1273 $\pm$ 74 vs. fasted, 1395 $\pm$ 96 nmol/liver). However, as in the nonperfused liver, there was a significant increase in the hepatic carnitine content of fasted-infected rats vs. that of fasted controls (2599 $\pm$ 235 vs. 1395 $\pm$ 96 nmol/liver). When octanoic acid was infused, the only significant increase was in the short-chain acylcarnitine fraction (2).

Long-chain acyl-CoA concentration increased in the fasted group compared to fed, while both long-chain acyl-CoA and acetyl-CoA concentrations decreased in livers from infected rats compared t. fasted control rats (Table I). When expressed on a total liver basis there was a significant decrease in the total CoA concentration by 48 h in livers from infected rats (fed, 1291 $\pm$ 124; fasted, 1325 $\pm$ 86; infected, 930 $\pm$ 57 nmol/liver). The concentrations of free CoA, acetyl-CoA and long-chain acyl-CoA were decreased in the livers from infected rats that were perfused with oleic acid compared to fasted perfused controls. Total coenzyme A decreased during infection (fed, 2139 $\pm$ 222; fasted, 1436 $\pm$ 126; fasted-infected, 648 $\pm$ 147 nmol/perfused liver).

In this study only about 6% of the total cellular carnitine was mitochondrial and approximately 20-40% of the mitochondrial carnitine

was long-chain acylcarnitine (Table II). The mitochondrial carnitine pool size was not significantly altered during infection compared to fasted controls. However, total cytosolic carnitine was significantly elevated in livers from the infected rats compared to fasted-controls. Both free carnitine and short-chain acylcarnitine were increased in fasted rats compared to fed rats, while long-chain acyl derivatives decreased in fasted-infected rats compared to fasted controls. Values for the total carnitine present in homogenates were 85-100% of those obtained by direct measurement of frozen intact tissue.

Between 23 and 31% of the total cellular CoA was associated with the mitochondria and 8-18% of this CoA was the long-chain derivative (Table II). There was a 50% decrease in the mitochondrial CoA pool size during infection. This decrease was observed in both free CoA and acetyl-CoA while no change occured in the long-chain acyl derivative. Cytosolic CoA also decreased in liver from infected rats compared to fasted controls. The change in cytosolic CoA was mainly due to decreases in the free CoA concentration. Short-chain acyl-CoA increased in the cytosolic space. In contrast to the recovery of carnitine, only 50-70% of the total CoA was recovered in assays of subcellular fractions. However, measurements of total CoA in freshly prepared homogenates were equal to 90-100% of the CoA present in intact tissue.

Fig. 1 shows isopycnic sucrose density profiles of carnitine, CoA, and marker enzymes from fasted control and fasted-infected rat liver. Catalase and glutamate dehydrogenase, respectively, mark the peroxisomal and mitochondrial peaks (bottom panels). Carnitine had a scattered sedimentation pattern with no significant change in concentration during infection. Eighty percent of the coenzyme was associated with the mitochondria as determined by the distribution of marker enzymes and

protein. CoA decreased by 50% in fractions prepared from livers of infected rats compared to fasted rats.

Acetylation and acylation states of carnitine and CoA. The carnitine acylation ratio reflects the direction of hepatic fat metabolism. This ratio (Table III) was increased by 30% in fasted rats and decreased in infected rats. The carnitine acetylation and CoA acylation ratios were the same for fasted and fed rats. However, the carnitine acetylation ratio was 2-fold greater in the fasted-infected group compared to fasted-controls. In the 48-h fasted rat, the CoA acetylation ratio was increased 67% above the fed and fasted-infected values.

Calculated molar concentrations of carnitine and CoA. The calculated molar concentrations of carnitine and CoA in cytosolic and mitochondrial matrix spaces shown in Table IV were based on the following assumptions and corrections: (a) 1 g fresh liver contains 0.4 ml cytosolic water (31) and 0.048 ml mitochondrial water (32); (b) although liver weight increases during infection, the percent of water per gram of liver does not change significantly (8); and (c) leakage of the mitochondrial marker enzyme, glutamate dehydrogenase, into the cytosolic fraction can be used to correct for mitochondrial breakage.

The molar concentration of total carnitine was higher in the cytosol than in the matrix while the molar concentrations of CoA were higher in the mitochondrial matrix than in the cytosol. The molar concentration of total mitochondrial CoA in the liver from fasted-infected rats was approximately half that found in liver from fed and fasted controls. Acetyl-CoA in fed and fasted controls had a 40-50 times higher molar concentration in mitochondria compared to cytosol, while this ratio was markedly decreased during infection. The concentration

of CoA decreased in both compartments during infection, with the greatest decrease observed in free CoA content.

Effect of infection on the redox state and on the availability of oxidizable substrates. Hepatic lactate and pyruvate concentrations were significantly increased in 48-h fasted-infected rats compared to fasted controls (Table V). The cytoplasmic $[NAD^+]/[NADH]$ ratio calculated from these results was not significantly altered by infection. However, there was a significant increase in the mitochondrial $[NAD^+]/[NADH]$ ratio during infection compared to fasting. This ratio change appears to reflect changes in both hepatic β-hydroxybutyrate and acetoacetate concentrations (16). The ratios of the nicotinamide nucleotide couples in rat liver are in agreement with those of Gumaa et al. (19) for fed and fasted rats. The concentration of α-glycerophosphate was slightly decreased in liver from both fasted and fasted-infected rats, while malate was increased in the liver from the infected rat compared to fasted controls. The concentration of dihydroxyacetone phosphate was not significantly altered by the infection. As previously reported (1), hepatic malonyl-CoA was not significantly different in fasted-infected rats compared to fasted controls.

Using established calculation methods (19), the concentration of citrate, oxaloacetate and malate and changes in the energy state were attributed to the cytosolic and mitochondrial compartments. Table VI shows that total citrate, oxaloacetate and malate are increased during the infection. The increases are due to significant increases in cytosolic concentrations of these metabolities. The mitochondrial pool sizes of citrate and malate decrease during the infection while that of oxaloacetate increases.

DISCUSSION

The inability of the infected rat to "ketone adapt" during starvation is due to a reduced hepatic ketogenic capacity (1, 2). Many systems have been used to study the mechanisms responsible for the regulation of fatty acid oxidation and ketone body formation (33, 34), but the factors responsible for the decreased ketogenesis associated with bacterial infection are not fully understood. Among the possible factors involved in control of hepatic ketogenesis are: (a) the supply of free fatty acids to the liver; (b) the proportion of free fatty acids that undergo oxidation as opposed to esterification; and (c) the availability of alternative substrates for oxidation (35).

When perfused with an equal concentration of long-chain fatty acids, similar amounts were taken up by liver from control and infected rats (2). Therefore, while availability of fatty acid precursor for ketone synthesis is undoubtedly a requirement for the ketotic state, it is not the only factor important in determining the rate of ketogenesis during infection. Earlier perfusion studies (1, 2) suggested that livers from infected rats were less efficient at oxidizing long-chain fatty acid and that more of the delivered fatty acid was esterified.

The degree of acylation of carnitine reflects the distribution of fatty acids between oxidation and esterification (36, 38). This ratio is increased in starved rats and reduced during feeding (36). Further, Kondrup and Grunnet (37) have shown a decreased acylation ratio in rats subjected to acute ethanol treatment, which is associated with a state of decreased fat oxidation. In the present studies the carnitine acylation ratio decreased in the fasted-infected rat suggesting a decreased rate of fatty acid oxidation. Thus, the inherent ability of the liver to direct fatty acids into oxidative pathways or toward triglyceride

synthesis is an important regulatory mechanism for controlling the rate of ketone body formation. This regulatory process could be altered by factors which influence the enzymes that direct acyl CoA into oxidative or esterification pathways.

Exton et al. (38) described the antiketogenic effect of lactate in fasted rats and showed that this effect was mimicked by pyruvate. Thus, an increase in both lactate and pyruvate concentrations in the fasted-infected rat liver (Table V) could be reflecting a decreased oxidation of fatty acids and an increased esterification to triglyceride and phospholipids. These observations are also consistent with the increased incorporation of long-chain fatty acids into liver lipids during perfusion of livers from infected rats with oleic acid (1) and the elevated plasma triglycerides (39, 40) observed during bacterial infection.

McGarry et al. (41) presented evidence that the hepatic mechanism involved in this metabolic adaptation is the carnitine-dependent transport of long-chain fatty acid. Both elevated carnitine (41) and decreased malonyl-CoA (15) concentrations correlated well with an increased hepatic ketogenic capacity. We have shown that, while livers from fasted rats conform to this model, livers from fasted-infected rats have elevated carnitine concentrations and malonyl-CoA levels equal to fasted-control values despite the reduced rate of ketogenesis (1). Also, when L-carnitine was added to the perfusate, it did not alter the rate of ketogenesis in liver from fasted-infected rats (2). These results suggested that in addition to being dependent on carnitine and malonyl-CoA concentr. ons, ketogenic adaptation must involve other controlling factors.

CoA and carnitine are key cofactors in determining rates of fatty acid utilization via lipogenic and oxidative pathways (42). The tissue

concentration and subcellular distribution of both of these cofactors and their long- and short-chain derivatives are, therefore, likely to influence the rates of the reciprocal pathways. One essential finding of the present study is that the concentration of CoA and its acyl and acetyl derivatives are significantly decreased during starvation plus infection. It has been suggested that the decrease in long-chain acyl-CoA in liver from S. pneumoniae-infected rats could be explained by the previous observation that circulating free fatty acids are decreased during this infection. However, both fed and fasted-infected livers perfused with oleic acid have long-chain acyl-CoA concentrations 50% that of fasted-control livers even though they were perfused with an equal amount of fatty acid. Therefore, the decrease in acyl-CoA derivatives appears to be an infection-related phenomenon not solely dependent on the availability of free fatty acid. Since triglycerides and long-chain acylcarnitines increase during infection (1), both pathways may be depleting a limited pool of long-chain acyl-CoA. Whether the decreased hepatic CoA concentration is reflecting a decreased synthesis, an increased release from hepatocytes, or an increased destruction of coenzyme A during infection can only be determined in future studies.

Since carnitine and CoA are compartmentalized within the liver cell, it is possible that the whole tissue measurements of these cofactors do not represent changes that occur at the active site of enzymes involved in fatty acid metabolism. Perturbations in the subcellular distribution of these cofactors were therefore investigated. The analyses performed on subcellular fractions aid in reconstructing the integrated functions of the cell (23). A number of limitations, nonetheless, exist when conclusions regarding the integrated cell function are made from the

subfraction analysis. To ensure subcellular integrity most isolations are performed in a buffered sucrose media which protects the cell from osmotic rupture and reduces leakage of internal enzymes and metabolites. In the present study recovery of total carnitine was essentially 100% in all fractions examined and was in good agreement with the concentrations reported by Parvin and Pande (42). However, the fractionation of liver homogenates reduced the recovery of added CoA, probably due to the fact that no reducing agent was added to the homogenate. Reducing agents were omitted from the fractionation media because it is not known what effect they might have on the other assay systems of interest. However, such agents were added to the fraction aliquot that was frozen for future assay of CoA. The absence of a reducing agent in the homogenizing media results in a considerable loss of CoA by disulfide exchange with denatured proteins (32), a problem which is of little concern when assaying freeze-clamped livers.

The percent of carnitine located in the matrix remained constant during the infection reflecting the 1:1 exchange system across the mitochondrial membrane (42). Although the total cellular CoA decreased during infection, the percent of total CoA associated with the mitochondrial and cytosolic spaces was not affected. Since β-oxidation is not altered by infection (2), the marked decrease in mitochondrial acetyl-CoA represents either an increased rate of utilization of this derivative for oxidation via the Krebs cycle or synthesis of ketones or a shuttling from the matrix space to the cytosolic compartment. Previous studies (1, 2) have indicated that the Krebs cycle activity is not increased and that ketogenesis is reduced in livers from fasted-infected rats. Thus, it would appear that the infectious process stimulates a shuttling of active acetyl groups away from ketogenesis and out of mitochondria to

the cytosolic compartment. The mitochondrial $[\text{acetyl-CoA}]^2/[\text{CoA}]$ ratio, estimates the concentration of acetoacetyl-CoA via the equilibrium of the acetoacetyl-CoA thiolase system of the mitochondrial matrix (34). This ratio is decreased during infection, which is further evidence for a direct relationship between the decreased matrix acetyl-CoA concentration and the reduced rate of ketogenesis.

While mitochondrial acetylation decreased, the cytosolic acetylation of carnitine and CoA increased during infection. Thus, these latter ratios are reflecting the increased accumulation of acetyl groups in the cytosol during the infection. Based on this concept, carnitine may be acting as a buffer system or "sink" for acetyl groups (43) being shuttled into the cytosol during infection. Since cytosolic acetyl-CoA was also increased during infection, more precursor could be available for the synthesis of new fatty acid or cholesterol, both of which have been shown to increase in pneumococcal infection (21, 39, 40, 42).

This apparent reciprocal relationship between lipogenesis and ketogenesis is governed not only by the rate of generation of acetyl-CoA from all precursors relative to its rate of disposal through ketogenic and nonketogenic pathways but also by the availability of alternative substrates for both pathways. Flux of acetyl groups through the Krebs cycle, coupled to long-chain fatty acid activation in the cytosol (44), provides a feedback mechanism which adjusts the cytosolic and mitochondrial pool sizes of acetyl-CoA, CoASH and carnitine. The availability of oxaloacetate for citrate synthase has been considered a regulatory factor in determining this flux of acetyl-CoA either into the Krebs cycle or into ketogenesis (44). Mitochondrial oxaloacetate concentrations increased slightly in fasted-infected rats. Thus, during infection, the increased concentrations of hepatic oxaloacetate

and malate appear to reflect a reduced ketogenic ability of the mitochondria, an increased synthesis of citrate and an increased flux through the gluconeogenic pathways. Indeed, gluconeogenesis has been shown to increase during an S. pneumoniae infection (45, 46); and pyruvate, although increased during infection, appears to be participating in gluconeogenic processes rather than contributing to the mitochondrial acetyl-CoA pool size.

The present study supports the concept that alterations in hepatic fatty acid metabolism and ketogenesis during infection may promote the continued use of amino acids for gluconeogenesis and therefore, contribute to the protein-wasting state that accompanies infectious illness. It can be concluded that infection-related decreases in ketogenesis from long-chain fatty acids are the result of an increased production of triglyceride from long-chain acyl groups, an increased shuttling of acetyl groups to the cytosol for synthetic processes and an increased transfer of acetyl groups from CoA to carnitine. Thus, necessary energy-yielding fuel, in the form of fatty acid, is directed away from oxidative pathways and ketogenesis toward pathways designed for the synthesis and storage of fat, establishing what might be considered a fatty-acid futile cycle in the liver of the infected host.

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Legend for Figure

FIGURE 1. Carnitine, CoA and marker enzymes in fractions after isopycnic sucrose density gradient centrifugation of the large granule fraction of liver homogenates from fasted and fasted-infected rats.

[1]Abbreviations: CoA, total coenzyme A; CoASH, free coenzyme A; CFU, colony-forming units; NAD, nicotinamide-adenine dinucleotide; NADH, nicotinamide-adenine dinucleotide, reduced; BW, body weight.

TABLE I

Carnitine and CoA Concentrations in Liver from Fed, Fasted and Fasted-Infected Rats

	Acid-insoluble	Acid-soluble		Total
		Short-chain	Free	
	nmol/g			
Carnitine				
Intact liver				
Fed	58 ± 6	47 ± 5	92 ± 8	198 ± 15
Fasted-control	88 ± 6	97 ± 13*	61 ± 6*	243 ± 10*
Fasted-infected	121 ± 8*†	184 ± 18*†	138 ± 9*†	452 ± 16†
Perfused liver				
Fed	84 ± 13	34 ± 4	51 ± 5	160 ± 8
Fasted-control	133 ± 11*	74 ± 8*	53 ± 4	265 ± 16*
Fasted-infected	205 ± 18*†	97 ± 16*†	78 ± 9	379 ± 16*†
CoA				
Intact liver				
Fed	26 ± 6	43 ± 5	76 ± 11	145 ± 12
Fasted-control	77 ± 8*	55 ± 5	74 ± 13	207 ± 11*
Fasted-infected	39 ± 11†	32 ± 4†	62 ± 8	130 ± 13†
Perfused liver				
Fed	50 ± 14	39 ± 6	117 ± 13	232 ± 25
Fasted-control	108 ± 14*	38 ± 4	133 ± 18	278 ± 29
Fasted-infected	46 ± 13†	11 ± 1*†	37 ± 8*†	96 ± 14*†

Values are expressed as mean ± SEM. Acetyl-CoA was the specific short-chain acyl-CoA derivative determined.

*$P < 0.01$ vs. fed.

†$P < 0.01$ vs. fasted-control.

TABLE II

Subcellular Distribution of Carnitine and CoA in Liver from Fed, Fasted and Fasted-Infected Rats

	Acid-insoluble	Acid-soluble		Total
		Short-chain	Free	
	nmol/g			
Carnitine				
Homogenate				
Fed	44 ± 7	46 ± 14	86 ± 21	229 ± 14
Fasted-control	88 ± 16*	75 ± 20	161 ± 13*	268 ± 29
Fasted-infected	44 ± 6†	155 ± 21*†	184 ± 10*	375 ± 24*†
Cytosolic				
Fed	32 ± 5	45 ± 11	75 ± 13	164 ± 15
Fasted-control	84 ± 16*	50 ± 18	144 ± 19*	211 ± 19*
Fasted-infected	44 ± 9†	104 ± 8*†	152 ± 8*	310 ± 10*†
Mitochondrial				
Fed	6 ± 1	1 ± 0.7	13 ± 2	16 ± 1
Fasted-control	7 ± 1	2 ± 0.9	11 ± 0.3	19 ± 1
Fasted-infected	5 ± 1	1 ± 0.4	12 ± 1	16 ± 1
CoA				
Homogenate				
Fed	7 ± 2	42 ± 4	56 ± 15	104 ± 15
Fasted-control	11 ± 1	26 ± 5*	103 ± 13*†	133 ± 14
Fasted-infected	8 ± 3	26 ± 4*	39 ± 3	76 ± 6
Cytosolic				
Fed	8 ± 1	26 ± 2	49 ± 16	82 ± 10
Fasted-control	9 ± 2	11 ± 4*	78 ± 9	102 ± 11
Fasted-infected	5 ± 3	23 ± 3†	37 ± 6*†	66 ± 8
Mitochondrial				
Fed	3 ± 1	11 ± 0.1	24 ± 5	38 ± 5
Fasted-control	2 ± 0.4	7 ± 1†	22 ± 3	30 ± 2†
Fasted-infected	3 ± 0.3	3 ± 1†	11 ± 1*†	17 ± 1

Values are mean ± SEM. Acetyl-CoA was the specific short-chain acyl-CoA derivative determined.

*$P < 0.01$ vs. fed.

†$P < 0.01$ vs. fasted-control.

TABLE III

Acetylation and Acylation Ratios of CoA and Carnitine in Rat Liver

	Fed	Fasted-control	Fasted-infected
$\frac{[\text{Acetyl-CoA}]}{[\text{Acetyl-CoA}][\text{CoASH}]}$	0.43 ± 0.04	0.72 ± 0.20*	0.43 ± 0.08†
$\frac{[\text{Acyl-CoA}]}{[\text{Acyl-CoA}][\text{CoASH}]}$	0.16 ± 0.04	0.11 ± 0.03	0.16 ± 0.03
$\frac{[\text{Acetylcarnitine}]}{[\text{Acetylcarnitine}][\text{Free carnitine}]}$	0.39 ± 0.03	0.40 ± 0.11	0.85 ± 0.08†
$\frac{[\text{Acylcarnitine}]}{[\text{Acylcarnitine}][\text{Free carnitine}]}$	0.27 ± 0.04	0.35 ± 0.04*	0.20 ± 0.03†

*$P < 0.05$ vs. fed.

†$P < 0.05$ vs. fasted-control.

TABLE IV

Calculated Molar Concentrations of CoA and Carnitine

	Metabolite	Cytosol	Mitochondrial matrix	M/C
		mM	mM	
Fed	CoA			
	Free	1.09	5.04	4.60
	Acetyl	0.06	2.30	38.30
	Long-chain	0.01	0.07	7.00
	Total	2.10	6.70	3.20
	Carnitine			
	Free	1.87	2.75	1.47
	Short-chain	1.13	0.02	0.02
	Long-chain	0.65	1.23	1.89
	Total	4.85	3.42	0.66
Fasted-control	CoA			
	Free	1.95	4.65	2.40
	Acetyl	0.02	1.45	48.30
	Long-chain	0.02	0.05	2.50
	Total	2.50	6.60	2.60
	Carnitine			
	Free	3.60	2.23	0.62
	Short-chain	1.25	0.04	0.03
	Long-chain	2.10	1.54	0.73
	Total	5.28	4.03	0.76
Fasted-infected	CoA			
	Free	0.93	2.23	2.40
	Acetyl	0.06	0.60	10.00
	Long-chain	0.01	0.06	6.00
	Total	1.60	3.50	2.20
	Carnitine			
	Free	3.80	2.60	0.62
	Short-chain	2.59	0.02	0.01
	Long-chain	1.09	0.98	0.90
	Total	7.76	3.38	0.44

TABLE V

Effects of S. pneumoniae infection on Hepatic Metabolites and Nucleotide Ratios

	Fed	Fasted-control	Fasted-Infected
		μmol/g	
Lactate	1.38 ± 0.10	0.33 ± 0.06*	0.67 ± 0.03*†
Pyruvate	0.088 ± 0.008	0.031 ± 0.003*	0.058 ± 0.003*†
β-Hydroxybutyric acid	0.17 ± 0.01	1.54 ± 0.07*	0.37 ± 0.02*†
Acetoacetic acid	0.09 ± 0.01	0.31 ± 0.02*	0.15 ± 0.01*†
Malate	0.45 ± 0.04	0.35 ± 0.08	0.54 ± 0.02
α-Glycerophosphate	0.21 ± 0.04	0.16 ± 0.03	0.18 ± 0.02
Dihydroxyacetone phosphate	0.012 ± 0.002	0.018 ± 0.004	0.016 ± 0.003
Malonyl-CoA	0.014 ± 0.003	0.005 ± 0.001*	0.003 ± 0.001*
$[NAD^+]/[NADH]$ Ratio			
Mitochondria	11.23 ± 1.45	4.26 ± 0.42	8.24 ± 0.45†
Cytoplasm	580 ± 55	965 ± 123	790 ± 57
$[NADP^+]/[NADPH]$ ratio			
Cytoplasm	0.007 ± 0.001	0.003 ± 0.001	0.004 ± 0.0002
[Acetyl-CoA]/[CoA] ratio			
Mitochondria	0.59 ± 0.15	1.08 ± 0.06*	0.32 ± 0.08†
Cytoplasm	1.26 ± 0.60	0.14 ± 0.04*	0.69 ± 0.10†
		M^{-1}	
$[ATP]/[ADP][HPO_4^{2-}]$	510	380	270

Values are mean ± SEM.

*$P < 0.01$ vs. fed.

†$P < 0.01$ vs. fasted-control.

TABLE VI

Distribution of Hepatic Citrate, Oxaloacetate (OAA) and Malate

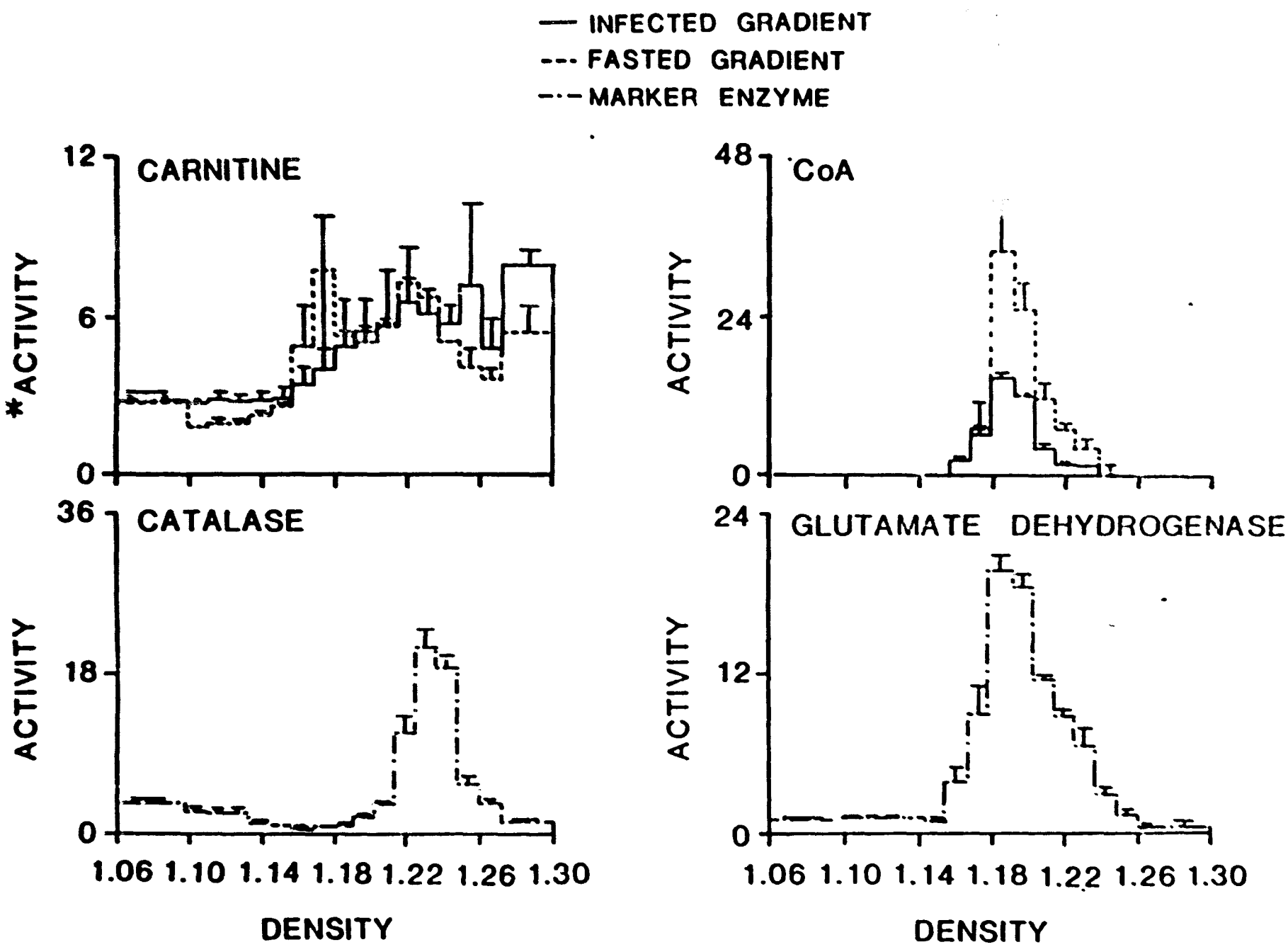
		Fed	Fasted-control	Fasted-infected
		nmol/g		
Citrate	Total	138 ± 12	55 ± 11*	208 ± 6*†
	Cytoplasm	111 ± 9	19 ± 4*	189 ± 5*†
	Mitochondria	22 ± 2	34 ± 7	19 ± 1
OAA	Total	7.37 ± 0.58	9.42 ± 0.27*	11.85 ± 0.34*†
	Cytoplasm	7.33 ± 0.58	9.4 ± 0.27*	11.82 ± 0.34*†
	Mitochondria	0.032 ± 0.003	0.019 ± 0.004	0.027 ± 0.002
Malate	Total	453 ± 36	351 ± 77	537 ± 15‡
	Cytoplasm	326 ± 27	210 ± 56	416 ± 12*†
	Mitochondria	127 ± 25	190 ± 34	121 ± 11

Values are mean ± SEM.

*P < 0.01 vs. fed.

†P < 0.01 vs. fasted-control.

‡P < 0.05 vs. fasted-control.



*ACTIVITY EXPRESSED AS U/ml